# EXHIBIT C

CII CANCER IMMUNOLOGY IMMUNOTHERAPY
OTHER BIOLOGICAL RESPONSE MODIFICATIONS

Referee B

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**Title:** Oral β-glucans enhance anti-tumor effects of monoclonal antibodies

## Referee's Comments to the Author:

This manuscript reports a potentially important observation in the development of β-glucan adjuvant therapy. Previous studies primarily in Japan had focused on fungal-derived β(1,3)-glucans that were given intravenously without a clear understanding of mechanism and with unpredictable outcome. Following up on reports that β-glucans functioned through a priming of leukocyte CR3 to kill tumor cells opsonized with the CR3-target ligand iC3b, the authors have not only confirmed the requirement for combination therapy with complement-activating mAbs, but have made the important discovery that the β-glucan could be given orally rather than intravenously, and that cereal grain-derived β(1,4)-glucans could be effective adjuvants with given orally instead of the more costly and difficult to obtain fungal β(1,3)-glucans that are generally given intravenously. Although future studies are required to define the mechanism of the oral route to confirm its requirement for leukocyte CR3, the current studies show that oral β(1,4)-glucan from barley is an effective adjuvant for three different mAbs in current clinical use that have much less effect on tumor growth when given individually (i.e., RituximAb, Herceptin, 3F8). Nevertheless, the finding that the oral barley β-glucan therapy requires the combined use of a complement-activating mAb and does not work either without mAb therapy or with a mAb that does not activate complement, is a strong indication that oral barley β-glucan functions similarly as i.v. fungal β-glucan in priming leukocyte CR3. Moreover, two previous publications have reported that barley β-glucan could prime leukocyte CR3 in vitro for cytotoxicity of tumor cells opsonized with iC3b, and that such cytotoxicity was CR3-dependent.

A few minor comments are provided to enhance the clarity of presentation.

Page 4, last paragraph: Barley β-glucans have not been shown to activate ADCC. Barley β-glucans have been shown to mediate NK cell CR3-dependent cytotoxicity, independently of ADCC. For example, in reference 11, NK cells incubated with barley β-glucan were shown to have an enhanced cytotoxicity of K562 cells that was blocked by OKM1 anti-CD11b, with no requirement for IgG or ADCC. Reference 44 showed that barley β-glucan would allow killing of NK-resistant tumor cells, provided that the target cells were opsonized with iC3b (again with no requirement for IgG or ADCC). Reference 42 has nothing to do with β-glucan and should be omitted.

Page 14, last sentence: Since these studies showed that even the most effective β-glucan required simultaneous administration of mAb, then the effectiveness of glucans or other agents such as wheat bran cannot be assessed in the absence of mAb co-administration. What this investigation shows is the necessity for co-administration of mAb for obtaining a therapeutic effect of β-glucan. It is important to note that previous studies that looked for a therapeutic effect of β-glucan administration did not incorporate co-administration of mAb as part of the protocol. It is likely that when β-glucans do function without co-administration of mAb, their tumor cytotoxic effect requires the presence of naturally-occurring antitumor Abs that target the tumors with iC3b.

Page 15: The requirement for Abs to deposit iC3b on tumors has been shown with yeast β-glucans given intravenously (49), and the reported requirement for T cells was shown to be the helper T cell function needed for generation of naturally-occurring anti-tumor Abs. Thus, the therapy was shown to be effective in T-cell deficient SCID or nude mice given natural Abs isolated from normal mouse serum (49). The bottom line is that all β-glucans (barley, fungi, seaweed) that function in tumor therapy have been shown to bind to CR3 in vitro and mediate leukocyte CR3-dependent cytotoxicity.